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(54) **ANTIBODY COMBINATION AGAINST REGENERATING ISLET-DERIVED PROTEIN 1? AND DETECTION KIT COMPRISING SAME**

(57) The present invention provides an antibody combination that can be used to detect regenerating islet-derived protein 1α (REGIA), each antibody in the antibody combination can specifically bind to REG1A with high affinity so as to form a double-antibody sandwich form, thereby enabling qualitative and quantitative detection of human REG1A. The antibody combination or an ELISA detection kit comprising the antibody combination can be used for auxiliary diagnosis or disease risk prediction of REG1A-related diseases.
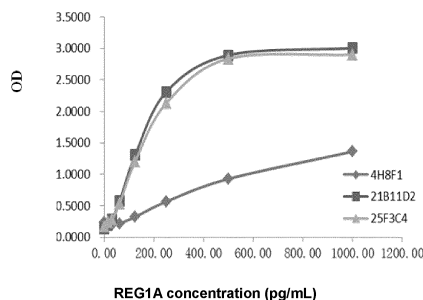
Fig. 2

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Description

5 **[0001]** For the right of priority of an application of Chinese Invention Patent (Application No.: CN202110798131.9) filed on July 14, 2021 claimed by the application of the present invention, all of its contents are incorporated herein by reference.

Field of the Invention

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[0002] The present invention relates to the field of biological testing. More particularly, the present invention relates to a biological testing technology of serologic markers, in particular to an antibody combination used for the quantitative detection of regenerating islet-derived protein 1α (REG1A) in serum and a detection kit containing REG1A.

15 **Background of the Invention**

[0003] The regenerating islet-derived protein 1α (regenerating family member 1 alpha, REG1A) is a member of re-generating gene (REG) family. The proteins of members of this family have some common structures, which mainly work in the digestive system, are closely related with the growth function of cells and may influence the growth of islet 20 cells, nerve cells and epithelial cells. Normally, REG1A is primarily expressed in the pancreas and is also observed in the gastric mucosa and the kidneys in small amounts. REG1A is not expressed in healthy intestines, but REG1A ex-pression will significantly rise if a lesion develops.
[0004] At present, extensive researches have been conducted into the associations between REG1A and different types of diseases. For example, researches show that human serum REG1A can be used as a clinical marker for early 25 detection of Alzheimer's disease; in addition, it is found that this gene is clinically significant in the development and progression of malignant tumors, such as nasopharyngeal carcinoma (NPC), bladder cancer, primary liver cancer, breast cancer, and cutaneous melanoma. More researches show that REG1A correlates with the development of inflammatory diseases of the gastrointestinal system and tumors.
[0005] A double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) has the following advantages: 30 easy to operate, high-efficiency, reliable and suitable for rapid screening of a large batch of samples, and especially a monoclonal antibody-based DAS-ELISA has higher stability and specificity. Therefore, attempts can be made to develop an antibody or an antibody combination that can specifically bind to REG1A and be effectively detected, to prepare a reagent for detection or diagnosis that can be used to detect the concentration of regenerating islet-derived protein 1α in serum qualitatively or quantitatively.

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Description of the Invention

[0006] To solve the technical problems, the present invention is to provide a new reagent and method that can be used to detect the existence or concentration of REG1A in serum with high sensitivity and specificity.
40 **[0007]** More particularly, the present invention is to provide an antibody combination targeting different epitopes on regenerating islet-derived protein 1α (REG1A). Each antibody in this combination needs to bind to REG1A with high affinity and specificity and form a double-antibody sandwich form to qualitatively or quantitatively detect human REG1A.
[0008] Based on the antibody combination, another object of the present invention is to provide an ELISA kit containing the antibody combination; for example, a DAS-ELISA kit is used for qualitative and quantitative detection of REG1A in 45 the samples to be tested.
[0009] The technical scheme of the present invention is as follows.
[0010] In one aspect, the prevent invention provides an antibody combination, and the antibody combination includes:

(i) Antibody 1: The Antibody 1 consists of heavy chains (HC) and light chains (LC), wherein the heavy chains include 50 a heavy chain CDR1 (H-CDR1) illustrated in SEQ ID NO. 1, a heavy chain CDR2 (H-CDR2) illustrated in SEQ ID NO. 2 and a heavy chain CDR3 (H-CDR3) illustrated in SEQ ID NO. 3 and the light chains include a light chain CDR1 (L-CDR1) illustrated in SEQ ID NO. 6, a light chain CDR2 (L-CDR2) illustrated in SEQ ID NO. 7 and a light chain CDR3 (L-CDR3) illustrated in SEQ ID NO. 8; and
(ii) Antibody 2: The Antibody 2 consists of heavy chains (HC) and light chains (LC), wherein the heavy chains include 55 a heavy chain CDR1 (H-CDR1) illustrated in SEQ ID NO. 11, a heavy chain CDR2 (H-CDR2) illustrated in SEQ ID NO. 12 and a heavy chain CDR3 (H-CDR3) illustrated in SEQ ID NO. 13 and the light chains include a light chain CDR1 (L-CDR1) illustrated in SEQ ID NO. 16, a light chain CDR2 (L-CDR2) illustrated in SEQ ID NO. 17 and a light chain CDR3 (L-CDR3) illustrated in SEQ ID NO. 18.

[0011] In the context of the present invention, the Antibody 1 and the Antibody 2 are the binding agents that can specifically bind the regenerating islet-derived protein 1α (REG1A).

[0012] Preferably, the heavy chains of the Antibody 1 include a heavy chain variable region (VH), the light chains include a light chain variable region (VL), and the VH of the Antibody 1 includes an amino acid sequence illustrated in SEQ ID NO. 4 and the VL includes an amino acid sequence illustrated in SEQ ID NO. 9; and/or, the heavy chains of the Antibody 2 include a heavy chain variable region (VH), the light chains include a light chain variable region (VL), and the VH of the Antibody 2 includes an amino acid sequence illustrated in SEQ ID NO. 14 and the VL includes an amino acid sequence illustrated in SEQ ID NO. 19.

[0013] Preferably, the Antibody 1 and the Antibody 2 are selected from mouse, chimeric and humanized antibodies. Preferably, the Antibody 1 and the Antibody 2 are selected from monoclonal, Fab, Fv and ScFv antibodies, etc.

[0014] Preferably, in the antibody combination provided in the present invention, the Antibody 1 and/or the Antibody 2 is a monoclonal antibody, consisting of 2 heavy chains and 2 light chains, and a mouse IgG1/Kappa isotype is preferred. In this aspect, the antibody structure is schematically illustrated in Fig. 1.

[0015] According to the Detailed Description of the Preferred Embodiments of the present invention, the heavy chains of the Antibody 1 include an amino acid sequence illustrated in SEQ ID NO. 5 and the light chains include an amino acid sequence illustrated in SEQ ID NO. 10; and/or, the heavy chains of the Antibody 2 include an amino acid sequence illustrated in SEQ ID NO. 15 and the light chains include an amino acid sequence illustrated in SEQ ID NO. 20.

[0016] According to the Detailed Description of the Preferred Embodiments of the present invention, the Antibody 1 and/or the Antibody 2 has a detectable label. Preferably, the Antibody 2 has a detectable label. The detectable label includes enzyme label, radioactive label, luminescent label, color rendering label, hapten (e.g. digoxin and biotin), metal complex or metal (e.g. colloidal gold).

[0017] In a further aspect, the present invention provides a use of the antibody combination to prepare a reagent for detecting the regenerating islet-derived protein 1α (REG1A). Alternatively, the present invention provides a use of the antibody combination to prepare a reagent for auxiliary diagnosis or disease risk prediction of REG1A-related diseases. Preferably, in the context of the present invention, the regenerating islet-derived protein 1α (REG1A) is a human regenerating islet-derived protein 1α (NCBI reference sequence: NP_002900.2).

[0018] In the context of the present invention, REG1A expression rises in the REG1A-related diseases. Preferably, the REG1A-related diseases are diseases of the digestive system. Preferably, the diseases are diseases of the digestive tract, such as inflammatory bowel disease (IBD), peptic ulcer, adenomatous polyp, Stage 1, Stage 2, Stage 3 or Stage 4 colorectal cancer, gastroenteritis or gastric cancer. Alternatively, the diseases are pancreatitides, such as acute pancreatitis.

[0019] In the context of the present invention, the reagent may be a reagent that can be used to detect the existence or concentration of REG1A in the biological samples from subjects. In the context of the present invention, "concentration" refers to the amount of the protein that can be detected in a biological sample, and "concentration" and "level" or "amount" can be used interchangeably herein. According to the Detailed Description of the Preferred Embodiments of the present invention, the existence or concentration of REG1A can be detected in whole blood, serum or plasma.

[0020] The detection results can be used to help diagnose whether the subjects suffer from any of the REG1A-related diseases or to predict whether the subjects are at risk of suffering from any of the REG1A-related diseases. In the context of the present invention, the subjects are mammals, preferably primates and more preferably humans. The biological samples are taken from one or more of the whole blood, plasma, serum, blood cell, ascites, lymph fluid, saliva, phlegm, perspiration, urine, mucus, interstitial fluid, tissue biopsy and cells from subjects, preferably whole blood, serum or plasma.

[0021] Preferably, the reagent can be used in the following detection methods: chemiluminescence immunoassay, immunonephelometry, enzyme-linked immunosorbent assay (ELISA), Western blotting, antibody microarray, immunoprecipitation, radioimmunoassay (RIA), etc. Preferably, the reagent is a detection reagent used for an ELISA, such as the detection reagent for a DAS-ELISA, wherein the ELISA is used to detect the regenerating islet-derived protein 1α (REG1A) in whole blood, serum or plasma. Preferably, in the ELISA, the Antibody 1 is used as a capture antibody (coated antibody) and the Antibody 2 is used as a detection antibody.

[0022] In still a further aspect, the present invention also provides a kit, and the kit contains the antibody combination in the present invention.

[0023] The kit is used to detect the existence or concentration of REG1A in biological samples from subjects, so it can be used for auxiliary diagnosis or disease risk prediction of REG1A-related diseases. Preferably, the regenerating islet-derived protein 1α (REG1A) is a human regenerating islet-derived protein 1α. The subjects are mammals, preferably primates and more preferably humans. The biological samples are taken from one or more of the whole blood, plasma, serum, blood cell, ascites, lymph fluid, saliva, phlegm, perspiration, urine, mucus, interstitial fluid, tissue biopsy and cells from subjects, preferably whole blood, serum or plasma. REG1A expression rises in the REG1A-related diseases. Preferably, the REG1A-related diseases are diseases of the digestive system. Preferably, the diseases are diseases of the digestive tract, such as inflammatory bowel disease (IBD), peptic ulcer, adenomatous polyp, Stage 1, Stage 2, Stage 3 or Stage 4 colorectal cancer, gastroenteritis or gastric cancer. Alternatively, the diseases are pancreatitides, such as

acute pancreatitis.

[0024] The kit can be used in the following detection methods: chemiluminescence immunoassay, immunonephelometry, enzyme-linked immunosorbent assay (ELISA), Western blotting, antibody microarray, immunoprecipitation, radio-immunoassay (RIA), etc. Preferably, the kit is an ELISA kit, such as DAS-ELISA kit, and the kit includes the antibody combination provided in the present invention. Preferably, the ELISA kit includes the Antibody 1 that is used as a capture antibody and the Antibody 2 that is used a detection antibody. Preferably, the Antibody 2 has a detectable label. The detectable label includes enzyme label, radioactive label, luminescent label, color rendering label, hapten (e.g. digoxin and biotin), metal complex or metal (e.g. colloidal gold).

[0025] More preferably, the kit also includes other reagents necessary for using ELISA to detect the existence or concentration of REG1A in biological samples. For example, the kit also includes one or more of the following items or all of the following items: REG1A calibrator and/or control material, antibody diluent, wash buffer, blocking buffer, diluent of the detected sample, ELISA plate, color-developing solution and stop buffer.

[0026] In one aspect, the present invention provides a method for detecting the regenerating islet-derived protein 1α (REG1A) in the biological samples taken from subjects, and the method includes: using the antibody combination or the kit provided in the present invention to detect the existence or concentration of REG1A in the biological samples from subjects.

[0027] Alternatively, the present invention provides a method for auxiliary diagnosis or disease risk prediction of REG1A-related diseases, and the method includes: using the antibody combination or the kit provided in the present invention to detect the concentration of REG1A in the biological samples from subjects. Additionally, the method includes: comparing the detected concentration of REG1A with a reference level, and a higher concentration of REG1A in the biological samples indicates that the subjects suffer from a REG1A-related disease or are at risk of suffering from a REG1A-related disease.

[0028] In this aspect, regenerating islet-derived protein 1α, REG1A-related diseases, subjects, biological samples, detection methods, etc. are defined above.

[0029] In the method provided in the present invention, the reference level refers to the value that is determined by comprehensively considering the ROC analysis and clinical needs of healthy population and patients in terms of the concentration of regenerating islet-derived protein 1α (REG1A) in the biological samples. According to the Detailed Description of the Preferred Embodiments of the present invention, the biological samples are serum, with the reference level of 60ng/ml.

[0030] According to the Detailed Description of the Preferred Embodiments of the present invention, the method uses the ELISA kit provided by the present invention, such as a DAS-ELISA kit. According to the Detailed Description of the Preferred Embodiments of the present invention, the following steps can be included:

(1) To coat a capture antibody (Antibody 1) on a ELISA plate and block it;
(2) To dilute the sample to be detected;
(3) To separately add the sample to be detected to different wells on the ELISA plate, optionally add a calibrator and/or a control material, add a labelled detection antibody (Antibody 2), and incubate it;
(4) To wash the plate;
(5) To detect the label on the detection antibody, such as color developing;
(6) To stop the reaction;
(7) To read the results, such as OD value; and
(8) To use the calibrator concentration as the x-axis and use the detection results (e.g. OD value) as the y-axis to draw a standard curve, and then calculate the concentration of REG1A in samples based on the detection results of the samples to be detected.

[0031] More particularly, the present invention provides an antibody combination targeting the human regenerating islet-derived protein 1α (REG1A). Each antibody in this combination can bind to REG1A with high affinity and specificity and bind to different epitopes of this protein, to form a double-antibody sandwich form to detect REG1A qualitatively or quantitatively in a combination. Correspondingly, the present invention also provides an ELISA method of using this antibody combination and its corresponding detection kit.

[0032] At present, clinical diagnosis of digestive system diseases is still dominated by pathological diagnosis. For example, the carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA19-9) known in the art can be used as the biomarkers for colorectal cancer and the carbohydrate antigen 72-4 (CA72-4) can be used as the biomarker for gastric cancer, but the definitive diagnosis of colorectal cancer and gastric cancer still needs to rely on colonoscopy, enteroscopy, gastroscopy, ultrasonography, imaging and pathological examination.

[0033] Through a lot of preliminary researches, the inventor of the present invention finds that this protein, REG1A, correlates with multiple diseases of the digestive system, such as colorectal cancer, adenomatous polyp, gastric cancer, IBD, and peptic ulcer, so accurate detection can be realized according to the level of REG1A in the biological samples

for auxiliary diagnosis or disease risk predication of these diseases. In this aspect, experiments have proven that the pairing of Antibody 1 (capture antibody) and Antibody 2 (detection antibody) provided in the present invention can qualitatively or quantitatively detect the human REG1A in serum with high accuracy and specificity. Particularly, compared with other antibody combinations, the optimal range of linearity of the human REG1A detected exceeds 1,000pg/mL in case of a combination of Antibody 1 and Antibody 2 provided in the present invention, and after human serum is diluted, the concentration of REG1A in serum can be effectively determined, and for a sample with an extremely high or low concentration, the sample dilution can be adjusted to ensure the accuracy of measurement results, so the requirements for accurately detecting the level of REG1A in samples can be fully met.

[0034] The inventor of the present invention has used a lot of serum samples taken from the patients with IBD, peptic ulcer, adenomatous polyp, Stage 1~ Stage 4 colorectal cancer, gastroenteritis, gastric cancer and acute pancreatitis for verification. The results show that if the antibody combination provided by the present invention is used in DAS-ELISA, the total sensitivity of detection of serum samples from patients can reach 75%, with the maximum value of 100%, and the detection results are very accurate; and for the serum samples taken before and after the effective treatment of REG1A-related diseases, the antibody combination provided in the present invention can be used to accurately detect the concentration of REG1A in serum samples and effectively monitor the changes in the concentration of REG1A in serum.

[0035] Thus, it is proved that the antibody combination and the ELISA (e.g. DAS-ELISA) method and kit based on this combination in the present invention have a good clinical application value. For example, the antibody combination, detection method and kit provided in the present invention can help doctors judge whether a disease is a functional lesion (e.g. flatulence and diarrhea) or an organic lesion (e.g. polyp, adenoma, gastrointestinal cancer, gastric ulcer, and IBD, which need to be further confirmed with gastrointestinal endoscopy); the same can be used for companion diagnostics/prognostic monitoring of REG1A-related diseases, with the aim to help diagnose whether the therapeutic drugs of patients are effective and repeated colonoscopies can be avoided; and the same can also be used to screen the gastrointestinal organic lesions of healthy population (e.g. gastric cancer and colorectal cancer).

Description of Figures

[0036] Combined with drawings, the embodiments provided in the present invention are explained in detail hereunder, wherein:

Fig. 1 is a schematic diagram of the structure of an antibody in the combination provided in the present invention when the antibody is a monoclonal antibody.
Fig. 2 illustrates the results of antibody pairing and screening.
Fig. 3 illustrates the results of antibody pairing and screening.
Fig. 4 is a ROC curve drawn according to the detection results of REG1A from different serum samples, wherein: 4-1: patients with Stage 1 colorectal cancer; 4-2: patients with Stage 2 colorectal cancer; 4-3: patients with Stage 3 colorectal cancer; 4-4: patients with Stage 4 colorectal cancer; 4-5: all patients with colorectal cancer; 4-6: patients with gastric cancer; 4-7: patients with gastroenteritis; 4-8: patients with adenomatous polyp; 4-9: patients with IBD; 4-10: patients with peptic ulcer; 4-11: patients with acute pancreatitis.
Fig. 5 illustrates the concentrations of REG1A in serum before and after the effective treatment of 29 patients with active IBD (****, $P < 0.001$).

Detailed Description of the Preferred Embodiments

[0037] The present invention is described by reference to the preferred embodiments. Those skilled in the art can understand that these embodiments are intended to illustrate the present invention and not to limit the range of the present invention in any way.

[0038] The experiment methods in the following embodiments are conventional methods unless otherwise specified. Raw materials and reagent materials used in the following embodiments are commercially available off-the-shelf (COTS) items unless otherwise specified.

[0039] The recombinant human REG1A is recombined and prepared according to NCBI reference sequence (NP_002900.2).

Embodiment 1 Acquisition of a monoclonal antibody for the human regenerating islet-derived protein 1α (REG1A)

[0040] Immunization: Mice are immunized by giving them a multipoint subcutaneous injection of a recombinant human REG1A, and this step is repeated for 3-4 times. After immunization, a small amount of blood is taken from these mice for potency assay and the spleens of mice with high potency are selected for further fusion.

[0041] Cell fusion: Splenocytes from mice are selected, grinded, and screened through a cell strainer, and then are centrifuged and washed with a culture solution. In this way, splenic lymphocyte suspension is obtained. After that, myeloma cells and splenocytes are mixed proportionally, and a fusion agent is added to the mixture for fusion.

[0042] Screening: A HAT selection culture solution is used for culturing, to screen out hybridoma cells, and meanwhile, the supernatant is measured and cultured with ELISA, to screen out the hybridoma cells producing antibodies that can be used to specifically recognize the human REG1A. Then, the screened positive hybridoma cells are cloned with the limiting dilution method to get stable monoclonal hybridoma cells.

[0043] Antibody production: The selected hybridoma cells are taken for expanded culture, the cultured supernatant is taken and purified with affinity chromatography to get monoclonal antibodies.

[0044] A total of 5 monoclonal antibodies are obtained and are mouse IgG1/Kappa isotypes after examination, which are separately named in the present invention: antibody 4H8F1; antibody 12F12A10; antibody 11D11B11; antibody 21B11D2; and antibody 25F3C4. The antibody sequence is as follows:

(i) 4H8F1:

Heavy chain (SEQ ID NO. 5 (removing signal peptide); wherein, the heavy chain variable region is SEQ ID NO. 4; CDRs are SEQ ID NO. 1/SEQ ID NO. 2/SEQ ID NO. 3 in order):

MGRLTSSFLLLIVPAYVLS*QVTLKESGPGILQPSQTLSLTCSFSGFSLS*<u>*TSGMSV*</u>
<u>*G*</u>*WIRQPSGKGLEWLA*<u>*HIWWNDDKFYNPALKS*</u>*RLTISKDTSKNQIFLKIASVVTADSAT*
YYCARI<u>*EEGWFAY*</u>*WGQGTLVTVST*<u>AKTTPPSVYPLAPGSAAQTNSMVTLGCLVKGYF</u>
<u>PEPVTVTWNSGSLSSGVHTFPAVLQSDLYTLSSSVTVPSSTWPSETVTCNVAHPASS</u>
<u>TKVDKKIVPRDCGCKPCICTVPEVSSVFIFPPKPKDVLTITLTPKVTCVVVDISKDDPEV</u>
<u>QFSWFVDDVEVHTAQTQPREEQFNSTFRSVSELPIMHQDWLNGKEFKCRVNSAAFP</u>
<u>APIEKTISKTKGRPKAPQVYTIPPPKEQMAKDKVSLTCMITDFFPEDITVEWQWNGQP</u>
<u>AENYKNTQPIMDTDGSYFVYSKLNVQKSNWEAGNTFTCSVLHEGLHNHHTEKSLSH</u>
<u>SPGK</u>

Light chain (SEQ ID NO. 10 (removing signal peptide); wherein, the light chain variable region is SEQ ID NO. 9, CDRs are SEQ ID NO. 6/SEQ ID NO. 7/SEQ ID NO. 8 in order):

MKSQTQVFVFLLLCVSAAHG*SIVMTQTPKFLLVSVGDRVTITC*<u>*KASQTMSNDVA*</u>
WYQQKPGQSPKLLIYY<u>*ASNRYT*</u>*GVPDRFTGSGYGTDFTFTISTVQAEDLAVYFC*<u>*QQD*</u>
<u>*YSSPLT*</u>*FGAGTKLELK*<u>RADAAPTVSIFPPSSEQLTSGGASVVCFLNNFYPKDINVKWKI</u>
<u>DGSERQNGVLNSWTDQDSKDSTYSMSSTLTLTKDEYERHNSYTCEATHKTSTSPIVK</u>
<u>SFNRNEC</u>

(ii) 12F12A10:

Heavy chain (SEQ ID NO. 15 (removing signal peptide); wherein, the heavy chain variable region is SEQ ID NO. 14; CDRs are SEQ ID NO. 11/SEQ ID NO. 12/SEQ ID NO. 13 in order):

MERHWIFLFLLSITAGVHS*QVQLQQSATELARPGASVKMSCKASGYTFT*<u>*SYMM*</u>
<u>*H*</u>*WVKQRPGQGLEWIG*<u>*YINPSSGYTDYNQKFKD*</u>*KTTLTADKSSSTAYMQLSSLTSEDS*
AVYYCAR<u>*YRYPHYFDY*</u>*WGQGTTLTVSS*AKTTPPSVYPLAPGSAAQTNSMVTLGCLV
KGYFPEPVTVTWNSGSLSSGVHTFPAVLQSDLYTLSSSVTVPSSTWPSETVTCNVAH
PASSTKVDKKIVPRDCGCKPCICTVPEVSSVFIFPPKPKDVLTITLTPKVTCVVVDISKD
DPEVQFSWFVDDVEVHTAQTQPREEQFNSTFRSVSELPIMHQDWLNGKEFKCRVN
SAAFPAPIEKTISKTKGRPKAPQVYTIPPPKEQMAKDKVSLTCMITDFFPEDITVEWQW
NGQPAENYKNTQPIMDTDGSYFVYSKLNVQKSNWEAGNTFTCSVLHEGLHNHHTEK
SLSHSPGK

Light chain (SEQ ID NO. 20 (removing signal peptide); wherein, the light chain variable region is SEQ ID NO. 19; CDRs are SEQ ID NO. 16/SEQ ID NO. 17/SEQ ID NO. 18 in order):

MDFQVQIFSFLLISASVIISRG*QIVLTQSPAIMSASPGEKVTMTC*<u>*SASSSVSYIH*</u>*W*
YQQKSGTSPKRWIY<u>*DTSKLAS*</u>*GVPARFSGSGSGTSYSLTISSMEAEDAAIYYC*<u>*QQWS*</u>
<u>*SNPPT*</u>*FGAGTKLELK*RADAAPTVSIFPPSSEQLTSGGASVVCFLNNFYPKDINVKWKI
DGSERQNGVLNSWTDQDSKDSTYSMSSTLTLTKDEYERHNSYTCEATHKTSTSPIVK
SFNRNEC

(iii) Antibody 11D11B11:

Heavy chain (SEQ ID NO. 21 (removing signal peptide)):

MSSEHRPLTMNFGLRLIFLVLTLKGVQC*DVKLVESGGGLVKPGGSLKLSCAAS*<u>*G*</u>
<u>*FTFSSFS*</u>*MSWFRQTPDKRLEWVAT*<u>*ISSGGSST*</u>*YYPDSVKGRFTISRDNAKNTLYLQM*
TSLKSEDTAIFYC<u>*RGGYYGNYDPLDY*</u>*WGQGTSVTVSA*AKTTAPSVYPLAPVCGDTTG
SSVTLGCLVKGYFPEPVTLTWNSGSLSSGVHTFPAVLQSDLYTLSSSVTVTSSTWPS
QSITCNVAHPASSTKVDKKIEPRGPTIKPCPPCKCPAPNLLGGPSVFIFPPKIKDVLMIS
LSPIVTCVVVDVSEDDPDVQISWFVNNVEVHTAQTQTHREDYNSTLRVVSALPIQHQ
DWMSGKEFKCKVNNKDLPAPIERTISKPKGSVRAPQVYVLPPPEEEMTKKQVTLTCM
VTDFMPEDIYVEWTNNGKTELNYKNTEPVLDSDGSYFMYSKLRVEKKNWVERNSYS
CSVVHEGLHNHHTTKSFSRTPGK

Light chain (SEQ ID NO. 22 (removing signal peptide)):

MGIKMESQTQVFVYMLLWLSGVDG*DIVMIQSQKFMSTSVGDRVSITCTAS*<u>*HNV*</u>

*DSN*VAWYQQKPGQSPQALIYS*SAS*YRYSGVPDRFTGSGSGTDFTLTINNVQSEDLAE

YFC*QQYNSYPLT*FGGGTRLEIKRADAAPTVSIFPPSSEQLTSGGASVVCFLNNFYPK

DINVKWKIDGSERQNGVLNSWTDQDSKDSTYSMSSTLTLTKDEYERHNSYTCEATH

KTSTSPIVKSFNRNEC

(iv) Antibody 21B11D2:

Heavy chain (SEQ ID NO. 23 (removing signal peptide)):

MEWSWVFLFLLSVIAGVQS*QAQLQQSGAELVRPGASVTLSCKAS*GYIFTDYEM

*HWVKQTPVHGLEWIGA*IDPETGGT*AYNQKFKGKARLTADKSSSTAYMELRSLTSEDS

*AVYYC*TIYYTNHFD*YWGQGTTLTVSS*AKTTPPSVYPLAPGCGDTTGSSVTLGCLVKG

YFPESVTVTWNSGSLSSSVHTFPALLQSGLYTMSSSVTVPSSTWPSQTVTCSVAHPA

SSTTVDKKLEPSGPISTINPCPPCKECHKCPAPNLEGGPSVFIFPPNIKDVLMISLTPKV

TCVVVDVSEDDPDVRISWFVNNVEVHTAQTQTHREDYNSTIRVVSALPIQHQDWMS

GKEFKCKVNNKDLPSPIERTISKIKGLVRAPQVYILPPPAEQLSRKDVSLTCLVVGFNP

GDISVEWTSNGHTEENYKDTAPVLDSDGSYFIYSKLDIKTSKWEKTDSFSCNVRHEG

LKNYYLKKTISRSPGK

Light chain (SEQ ID NO. 24 (removing signal peptide)):

MKLPVRLLVLMFWIPASSS*DVVMTQTPLSLPVSLGDQASISCRSS*QSLVYSNGD

*TY*LYWYLQKPGQSPKLLIY*KVS*NRFSGVPDRFSGSGSGTDFTLKISRVEAEDLGVYF

*CSQSTHVPLT*FGAGTKLELKRADAAPTVSIFPPSSEQLTSGGASVVCFLNNFYPKDIN

VKWKIDGSERQNGVLNSWTDQDSKDSTYSMSSTLTLTKDEYERHNSYTCEATHKTS

TSPIVKSFNRNEC

(v) Antibody 25F3C4

Heavy chain (SEQ ID NO. 25 (removing signal peptide)):

MEWSWVFLFLLAVIAGVQS*QVQLQQSGAELVRPGASVTLSCKAS**GYTFTDYEV** *HWVKQTPVHGLEWIGA**IDPETGGT**AYNLKFKGKAILTADKSSSTAYMELRSLTSEDSA* *VYYF**TIYYTNNFD**YWGQGTALTVSS*AKTTPPSVYPLAPGCGDTTGSSVTLGCLVKGY FPESVTVTWNSGSLSSSVHTFPALLQSGLYTMSSSVTVPSSTWPSQTVTCSVAHPAS STTVDKKLEPSGPISTINPCPPCKECHKCPAPNLEGGPSVFIFPPNIKDVLMISLTPKVT CVVVDVSEDDPDVRISWFVNNVEVHTAQTQTHREDYNSTIRVVSALPIQHQDWMSG KEFKCKVNNKDLPSPIERTISKIKGLVRAPQVYILPPPAEQLSRKDVSLTCLVVGFNPG DISVEWTSNGHTEENYKDTAPVLDSDGSYFIYSKLDIKTSKWEKTDSFSCNVRHEGL KNYYLKKTISRSPGK

Light chain (SEQ ID NO. 26 (removing signal peptide)):

MKLPVRLLVLMFWIPASSS*DVVMTQTPLSLPVSLGDQASISCRSS**QSLVYSDGN** *TY**LHWYLQKPGQSPKLLIY**KVS**YRFSGVPDRFSGSGSGTDFTLKISRVEAEDLGVYF* *CS**QSTHVPLT**FGAGTKLELK*RADAAPTVSIFPPSSEQLTSGGASVVCFLNNFYPKDIN VKWKIDGSERQNGVLNSWTDQDSKDSTYSMSSTLTLTKDEYERHNSYTCEATHKTS TSPIVKSFNRNEC

[0045] In the sequences above, the part in bold refers to a signal peptide; the part in italic type refers to a variable region, wherein the underlined part denotes a CDR; and the underlined part behind the variable region is a constant region.

Embodiment 2 Screening and verification of capture and detection antibodies (I) First pairing and screening

[0046] For the 5 monoclonal antibodies obtained in Embodiment 1, one antibody is paired with another as capture and detection antibodies, respectively, and the detection effect on the recombinant human REG1A of antigens is measured. The process is as follows:

Coating: A diluent (10mM pH 7.4 PBS) is used to dilute the capture antibody to 2.5pg/mL as a coating buffer, and the coating buffer is then added to the wells on an ELISA plate (100μL per well) to be incubated overnight at 4°C. After that, the liquid is removed from wells, and the plate is washed twice with a wash buffer (10mM pH7.4 PBS+0.5% Tween 20, PBST);

Blocking: A blocking buffer (10mM pH7.4 PBS+1 % BSA+0.5% Tween 20) is added to the wells on an ELISA plate (250μL per well) to be incubated for 2h at 37°C. Then, the liquid is removed from wells, and the plate is washed twice with PBST;

Antigen incubation: A basic diluent (10mM pH7.4 PBS+0.5% BSA+0.5% Tween 20) is used to prepare the recombinant REG1A into solutions of different concentrations, and then the solutions are added to the wells of an ELISA plate (100μL per well) to be incubated for 1h at 37°C. After that, the liquid is removed from wells, and the plate is washed 3 times with PBST;

Detection antibody incubation: A basic diluent is used to dilute the biotin-labeled detection antibody to 1μg/mL, and then the solution is added to the wells of an ELISA plate (100μL per well) to be incubated for 1h at 37°C. After that, the liquid is removed from wells, and the plate is washed 3 times with PBST;

Secondary antibody incubation: A basic diluent is used to dilute the HRP-labeled streptavidin (SIGMA) by a factor of 20,000, and then the solution is added to the wells of an ELISA plate (100μL per well) to be incubated for 45min at 37°CAfter that, the liquid is removed from wells, and the plate is washed 3 times with PBST;

Color developing: A TMB substrate solution (produced by Huzhou InnoReagents Co., Ltd.) is added to the wells of an ELISA plate (100μL per well) for color development for 15min at 37°C away from light. Then, a 50μL stop buffer (0.2M sulfuric acid) is added to stop the reaction;

Reading: A microplate reader is used to measure the OD value of each well at the dominant wavelength of 450nm and the sub-wavelength of 620/630nm (the OD value at the dominant wavelength of 450nm minus the OD value at the sub-wavelength of 620/630nm).

[0047]   The results are shown in Table 1.

Table 1. Results of the First Pairing Experiment (OD Value)

Capture antibody (2.5μg/mL)	Antigen (ng/mL)						Detection antibody (1μg/mL)
	100.00	33.33	11.11	3.70	1.23	0	
4H8F1	0.09	0.081	0.081	0.079	0.074	0.074	4H8F1-biotin
	4.242	4.223	4.104	3.736	2.465	0.21	11D11B11-biotin
	4.33	4.33	4.217	3.841	2.987	0.088	12F12A10-biotin
	0.08	0.073	0.07	0.068	0.072	0.069	21B11D2-biotin
	0.101	0.097	0.095	0.09	0.09	0.093	25F3C4-biotin
11D11B11	4.235	4.478	3.872	2.865	1.373	1.208	4H8F1-biotin
	0.064	0.064	0.072	0.066	0.066	0.079	11D11B11-biotin
	4.216	4.083	3.896	2.811	1.047	0.083	12F12A10-biotin
	0.065	0.063	0.066	0.06	0.07	0.068	21B11D2-biotin
	0.083	0.083	0.076	0.077	0.073	0.077	25F3C4-biotin
12F12A10	3.183	3.267	3.05	2.425	1.297	0.068	4H8F1-biotin
	3.153	3.028	2.688	1.543	0.934	0.075	11D11B11-biotin
	0.096	0.098	0.088	0.088	0.098	0.089	12F12A10-biotin
	3.352	3.21	2.892	2.384	1.177	0.068	21B11D2-biotin
	3.77	3.759	3.506	2.998	1.608	0.085	25F3C4-biotin
21B11D2	0.084	0.078	0.073	0.077	0.078	0.075	4H8F1-biotin
	0.075	0.077	0.077	0.069	0.089	0.074	11D11B11-biotin
	4.648	4.587	4.228	4.589	3.898	0.09	12F12A10-biotin
	0.068	0.071	0.069	0.069	0.066	0.073	21B11D2-biotin
	0.106	0.094	0.111	0.089	0.087	0.09	25F3C4-biotin
25F3C4	0.086	0.085	0.078	0.082	0.087	0.076	4H8F1-biotin
	0.089	0.083	0.079	0.076	0.071	0.074	11D11B11-biotin
	4.476	4.289	4.373	4.251	3.764	0.093	12F12A10-biotin
	0.073	0.071	0.069	0.068	0.066	0.068	21B11D2-biotin
	0.107	0.118	0.103	0.102	0.099	0.093	25F3C4-biotin

[0048]   According to Table 1, compared with other sets of paired antibodies, the following three sets of paired antibodies can obtain a better detection effect: separately used as 4H8F1, 21B11D2 and 25F3C4 of the capture antibody and as 12F12A10 of the detection antibody.

(II) Second pairing and screening

[0049]   The three sets of paired antibodies obtained from the first pairing and screening are further screened. The process is as follows:

Coating: A PBS is used to dilute the capture antibody to 1μg/mL as a coating buffer, and then the coating buffer is

added to the wells on an ELISA plate (100µL per well) to be incubated overnight at 4°C. After that, the liquid is removed from wells, and the plate is washed twice with PBST;

Blocking: A blocking buffer is added to the wells on an ELISA plate (250µL per well) to be incubated for 2h at 37°C. After that, the liquid is removed from wells, and the plate is washed twice with PBST;

Antigen incubation: A basic diluent is used to prepare the recombinant REG1A into solutions of different concentrations, and then the solutions are added to the wells of an ELISA plate (100µL per well) to be incubated for 1h at 37°CAfter that, the liquid is removed from wells, and the plate is washed 3 times with PBST;

Detection antibody incubation: A basic diluent is used to dilute the HRP-labeled detection antibody by a factor of 30,000, and then the solution is added to the wells of an ELISA plate (100µL per well) to be incubated for 1h at 37°C. After that, the liquid is removed from wells, and the plate is washed 3 times with PBST;

Color developing: A TMB substrate solution is added to the wells of an ELISA plate (100µL per well) for color development for 15min at 37°C away from light. Then, a 50µL stop buffer (0.2M sulfuric acid) is added to stop the reaction;

Reading: A microplate reader is used to measure the OD value of each well at the dominant wavelength of 450nm and the sub-wavelength of 620/630nm (the OD value at the dominant wavelength of 450nm minus the OD value at the sub-wavelength of 620/630nm).

[0050] The results are shown in Table 2 and Fig. 2.

Table 2. Results of the Second Pairing Experiment (OD Value)

Detection antibody		12F12A10		
Coated antibody		4H8F1	21B11D2	25F3C4
REG1A pg/mL	1000	1.3618	3.0054	2.9005
	500	0.9267	2.8918	2.8358
	250	0.5655	2.3096	2.1300
	125	0.3233	1.3078	1.2050
	62.5	0.2205	0.5776	0.5386
	31.25	0.1925	0.2769	0.2784
	15.625	0.2028	0.1943	0.2411
	0	0.2394	0.1382	0.1769

[0051] It can be seen from the results that the antibody pairs - 21B11D2-12F12A10 and 25F3C4-12F12A10 - show a good linearity within the range that the concentration of REG1A is less than 500pg/mL, and the antibody pair - 4H8F1-12F12A10 - shows the optimal linearity when the concentration exceeds 1,000pg/mL. Considering that the concentration of REG1A in human serum samples is Level ng, the antibody pair - 4H8F1-12F12A10 - is finally used as the capture and detection antibodies.

(III) Verification of capture and detection antibodies

[0052] 4H8F1, 21B11D2 and 25F3C4 are used separately used as coated antibodies and 12F12A10 is used as the detection antibody to test serum samples. The steps in "(II) Second pairing and screening" above are followed. When serum samples are tested, antigens are replaced with serum samples that are then added to the wells (100µL per well) directly. The concentration of proteins in serum samples relative to the standard curve are calculated.

[0053] The results are shown in Table 3 and Fig. 3.

Table 3. Results of Serum Detection of REG1A Antibody Pairs

Detection antibody		12F12A10		
Coated antibody		4H8F1	21B11D2	25F3C4
Standard curve	Standard protein concentration (ng/mL)	Value		
	100	2.8884	2.2908	2.6976
	50	2.6029	2.2536	2.6941
	10	2.2273	2.1506	2.6155
	1	1.4997	1.1509	1.6932
	0.1	0.6664	0.1889	0.1995
	0	0.0589	0.1013	0.0693
Serum samples	No.	Concentration (ng/mL)		
	170	84.0	4.1	2.3
	232	81.2	2.6	3.4
	242	43.9	2.9	6.3
	253	2.8	24.9	1.1

[0054]   It can be seen from the standard curve that the two antibody pairs - 21B11D2-12F12A10 and 25F3C4-12F12A10 - show a small change in the OD values when the antigen concentration is equal to or greater than 10ng/mL, speculating that the antigen-antibody response in this case has reached the saturation point. This point can also be demonstrated by the detection results of serum samples, and for the two antibody pairs, the measured concentrations of proteins in serum samples are not higher than 10ng/mL. Taking samples 232 and 242 as an example, the measured concentrations of proteins of these two samples - 4H8F1-12F12A10 - vary greatly, but the concentrations detected of the two antibody pairs - 21B11D2-12F12A10 and 25F3C4-12F12A10 - show no significant differences.

Embodiment 3 Adoption of the antibody pair - 4H8F1-12F12A10 - for measurement of REG1A in different samples

[0055]   The serum samples are taken from 805 normal cases and the serum samples from 1,924 patients with colorectal cancer (CRC) (including 344 patients with Stage 1 CRC, 669 patients with Stage 2 CRC, 567 patients with Stage 3 CRC and 344 patients with Stage 4 CRC), 89 patients with gastric cancer, 198 patients with adenomatous polyp, 34 patients with IBD, 80 patients with peptic ulcer, 158 patients with gastroenteritis and 7 patients with acute pancreatitis. These serum samples are obtained from Sun Yat-sen University Cancer Center (SYSUCC) and Zhujiang Hospital of Southern Medical University.

[0056]   As described above, the antibody combination (4H8F1-12F12A10) provided by the present invention is used to test serum samples, and 4H8F1 and 12F12A10 are used as a coated antibody (capture antibody) and a detection antibody respectively to prepare an ELISA kit. Normal cases are used as a control group whose data are compared with the data of the disease group, to conduct a ROC analysis. Then, the results of ROC analysis and the needs of clinical application are combined to determine the cutoff value of concentration of REG1A in human serum as 60ng/ml, and based on it, the corresponding sensitivity and specificity are calculated. The results are shown in Table 4.

Table 4. Detection of the Specificity and Sensitivity of Different Serum Samples (Unit of Protein Concentration: ng/ml)

Cutoff: 60	Mean protein concentration	T-test	AUC	P value	Total number of cases	Negative	Positive	Sensitivity	Specificity
Normal cases	46.42				805	671	134		83.35%
CRC 1	90.87	2.72E-28	0.9038	<0.0001	344	86	258	75.00%	
CRC 2	94.42	1.68E-41	0.9007	<0.0001	669	158	511	76.38%	
CRC 3	96.97	1.92E-42	0.9147	<0.0001	567	116	451	79.54%	
CRC 4	112.08	2.08E-19	0.8997	<0.0001	344	75	269	78.20%	
CRC ALL	96.36	1.04E-11 0	0.8946	<0.0001	1924	477	1447	75.21%	
Gastric cancer	146.42	6.25E-09	0.9033	<0.0001	89	19	70	78.65%	
Adenomatous polyp	76.85	1.27E-15	0.7672	<0.0001	198	77	121	61.11%	
IBD	103.16	1.41E-07	0.9059	<0.0001	34	8	26	76.47%	
Peptic ulcer	118.33	6.55E-10	0.8518	<0.0001	80	25	55	68.75%	
Gastroenteritis	102.38	3.28E-24	0.8705	<0.0001	158	39	119	75.32%	
Acute pancreatitis	334.58	3.87E-02	0.9807	<0.0001	7	0	7	100.00%	
							Total sensitivity	75.53%	

[0057] According to the measured data above, GraphPad Prism is used to draw a receiver operating characteristic (ROC) curve, and the results are shown in Fig. 4.

Embodiment 4 Adoption of the antibody pair - 4H8F1-12F12A10 - for measurement of REG1A in samples before and after the treatment of patients with inflammatory bowel disease (IBD)

[0058] The serum samples of 29 patients with IBD before and after the effective treatment of IBD are obtained from SYSUCC. Infliximab and other monoclonal antibodies or hormones that are often used clinically as a treatment for IBD are used to treat the patients with IBD. The confirmation of treatment efficacy is made by the attending physician according to the endoscopic score. The prevalence situation of patients with IBD is confirmed though enteroscopy, and in the absence of any therapeutic intervention, the serum samples before treatment are obtained. After patients receive medication for 3 months and the treatment is confirmed to be effective, the serum samples after treatment are obtained.

[0059] As described above, the antibody combination (4H8F1-12F12A10) provided by the present invention is used to test serum samples, and 4H8F1 and 12F12A10 are used as a coated antibody (capture antibody) and a detection antibody respectively to prepare an ELISA kit, and the concentrations of REG1A in serum samples before and after the effective treatment are tested.

[0060] Through the test of the serum samples taken from 29 patients with active IBD, it is found that the concentration of REG1A in serum of 26 patients (89.7%; $P < 0.0001$) after effective treatment is significantly reduced, and the median concentration in serum decreases from 85.86 ng/mL (31.68-317.80) before effective treatment to 60.20 ng/mL (29.34-171.50). For the results, see Fig. 5.

[0061] Experiments prove that the antibody pair - 4H8F1-12F12A10 - provided by the present invention can be used to conduct the companion diagnostics/prognostic monitoring of IBD and help confirm whether the therapeutic drugs for patients with IBD are effective to avoid repeated colonoscopies.

[0062] The foregoing description of the preferred embodiments of the present invention is not limited to the present invention. Those skilled in the field may make various changes or deformations according to the present invention, and all changes or deformations are therefore intended to be embraced in the Claims of the present invention, without departing from the spirit thereof.

Claims

1. An antibody combination, and the antibody combination comprising:

   (i) Antibody 1: The Antibody 1 consists of heavy chains (HC) and light chains (LC), wherein the heavy chains include a heavy chain CDR1 (H-CDR1) illustrated in SEQ ID NO. 1, a heavy chain CDR2 (H-CDR2) illustrated in SEQ ID NO. 2 and a heavy chain CDR3 (H-CDR3) illustrated in SEQ ID NO. 3 and the light chains include a light chain CDR1 (L-CDR1) illustrated in SEQ ID NO. 6, a light chain CDR2 (L-CDR2) illustrated in SEQ ID NO. 7 and a light chain CDR3 (L-CDR3) illustrated in SEQ ID NO. 8; and
   (ii) Antibody 2: The Antibody 2 consists of heavy chains (HC) and light chains (LC), wherein the heavy chains include a heavy chain CDR1 (H-CDR1) illustrated in SEQ ID NO. 11, a heavy chain CDR2 (H-CDR2) illustrated in SEQ ID NO. 12 and a heavy chain CDR3 (H-CDR3) illustrated in SEQ ID NO. 13 and the light chains include a light chain CDR1 (L-CDR1) illustrated in SEQ ID NO. 16, a light chain CDR2 (L-CDR2) illustrated in SEQ ID NO. 17 and a light chain CDR3 (L-CDR3) illustrated in SEQ ID NO. 18.

2. An antibody combination according to Claim 1, which is **characterized in that** the Antibody 1 and the Antibody 2 are the binding agents that can specifically bind to the regenerating islet-derived protein 1α (REG1A).

3. An antibody combination according to Claim 1, which is **characterized in that** the heavy chains of the Antibody 1 include a heavy chain variable region (VH), the light chains include a light chain variable region (VL), and the VH of the Antibody 1 includes an amino acid sequence illustrated in SEQ ID NO. 4 and the VL includes an amino acid sequence illustrated in SEQ ID NO. 9; and
   The heavy chains of the Antibody 2 include a heavy chain variable region (VH), the light chains include a light chain variable region (VL), and the VH of the Antibody 2 includes an amino acid sequence illustrated in SEQ ID NO. 14 and the VL includes an amino acid sequence illustrated in SEQ ID NO. 19.

4. An antibody combination according to Claim 3, which is **characterized in that** the Antibody 1 and the Antibody 2 are selected from mouse, chimeric and humanized antibodies.

5. An antibody combination according to Claim 3, which is **characterized in that** the Antibody 1 and the Antibody 2 are selected from monoclonal, Fab, Fv and ScFv antibodies.

6. An antibody combination according to Claim 5, which is **characterized in that** each of the Antibody 1 and the Antibody 2 is a monoclonal antibody, consisting of 2 heavy chains and 2 light chains.

7. An antibody combination according to Claim 6, which is **characterized in that** the Antibody 1 and the Antibody 2 are the mouse IgG1/Kappa isotypes.

8. An antibody combination according to Claim 6, which is **characterized in that** the heavy chains of the Antibody 1 include an amino acid sequence illustrated in SEQ ID NO. 5 and the light chains include an amino acid sequence illustrated in SEQ ID NO. 10; and, the heavy chains of the Antibody 2 include an amino acid sequence illustrated in SEQ ID NO. 15 and the light chains include an amino acid sequence illustrated in SEQ ID NO. 20.

9. An antibody combination according to any one of Claims 1 to 8, which is **characterized in that** the Antibody 1 or the Antibody 2 has a detectable label.

10. An antibody combination according to Claim 9, which is **characterized in that** the Antibody 2 has a detectable label.

11. An antibody combination according to Claim 10, which is **characterized in that** the detectable label is an enzyme label, radioactive label, luminescent label, color rendering label, hapten, metal complex or metal.

12. A use of the antibody combination to prepare a reagent for detecting the regenerating islet-derived protein 1α (REG1A) according to any one of Claims 1 to 11.

13. A use of the antibody combination to prepare a reagent for auxiliary diagnosis or disease risk prediction of REG1A-related diseases according to any one of Claims 1 to 11.

14. A use according to Claim 12 or 13, which is **characterized in that** the regenerating islet-derived protein 1α (REG1A) is a human regenerating islet-derived protein 1α.

15. A use according to Claim 13, which is **characterized in that** REG1A expression rises in the REG1A-related diseases.

16. A use according to Claim 15, which is **characterized in that** the REG1A-related diseases are diseases of the digestive system.

17. A use according to Claim 16, which is **characterized in that** the diseases of the digestive system are diseases of the digestive tract.

18. A use according to Claim 17, which is **characterized in that** the disease of the digestive tract is IBD, peptic ulcer, adenomatous polyp, Stage 1, Stage 2, Stage 3 or Stage 4 colorectal cancer, gastroenteritis or gastric cancer.

19. A use according to Claim 16, which is **characterized in that** the disease of the digestive system is pancreatitis.

20. A use according to Claim 19, which is **characterized in that** the pancreatitis is acute pancreatitis.

21. A use according to Claim 12 or 13, which is **characterized in that** the reagent can be used in the following detection methods: chemiluminescence immunoassay, immunonephelometry, enzyme-linked immunosorbent assay (ELISA), Western blotting, antibody microarray, immunoprecipitation, and radioimmunoassay (RIA).

22. A use according to Claim 21, which is **characterized in that** the reagent is a detection reagent used for ELISA.

23. A use according to Claim 22, which is **characterized in that** the detection reagent is a detection reagent used for DAS-ELISA; wherein the ELISA is used to detect the regenerating islet-derived protein 1α (REG1A) in whole blood, serum or plasma.

24. A use according to Claim 23, which is **characterized in that**, in the ELISA, the Antibody 1 is used as a capture antibody and the Antibody 2 is used as a detection antibody.

25. A kit that includes the antibody combination according to any one of Claims 1 to 11.

26. A kit according to Claim 25, which is **characterized in that** the kit is a kit used in the following detection methods: chemiluminescence immunoassay, immunonephelometry, enzyme-linked immunosorbent assay (ELISA), Western blotting, antibody microarray, immunoprecipitation, and radioimmunoassay (RIA).

27. A kit according to Claim 26, which is **characterized in that** the kit is an ELISA kit.

28. A kit according to Claim 27, which is **characterized in that** the kit is a DAS-ELISA kit.

29. A kit according to Claim 28, which is **characterized in that** the kit includes the Antibody 1 that is used as a capture antibody and the Antibody 2 that is used as a detection antibody.

30. A kit according to Claim 29, which is **characterized in that** the Antibody 2 has a detectable label.

31. A kit according to Claim 29, which is **characterized in that** the detectable label is an enzyme label, radioactive label, luminescent label, color rendering label, hapten, metal complex or metal.

32. A kit according to any one of Claims 25 to 31, which is **characterized in that** the kit also includes other reagents necessary for using ELISA to detect the existence or concentration of REG1A in biological samples.

33. A kit according to Claim 32, which is **characterized in that** the other reagents are one or more of the following items: REG1A calibrator and/or control material, antibody diluent, wash buffer, blocking buffer, diluent of the detected sample, ELISA plate, color-developing agent and stop buffer.

34. A method for the auxiliary diagnosis or disease risk prediction of REG1A-related diseases, including: using an antibody combination according to any one of Claims 1 to 11 or a kit according to any one of Claims 25 to 31 to detect the concentration of REG1A in biological samples from subjects.

35. A method according to Claim 34, which is **characterized in that** the method also includes: comparing the detected concentration of REG1A with a reference level, and a higher concentration of REG1A in the biological samples indicates that the subjects suffer from a REG1A-related disease or are at risk of suffering from a REG1A-related disease.
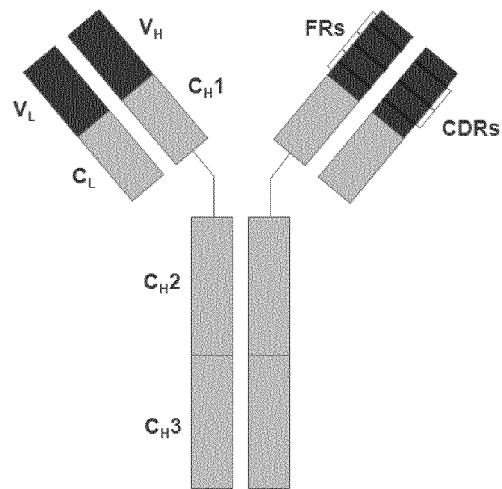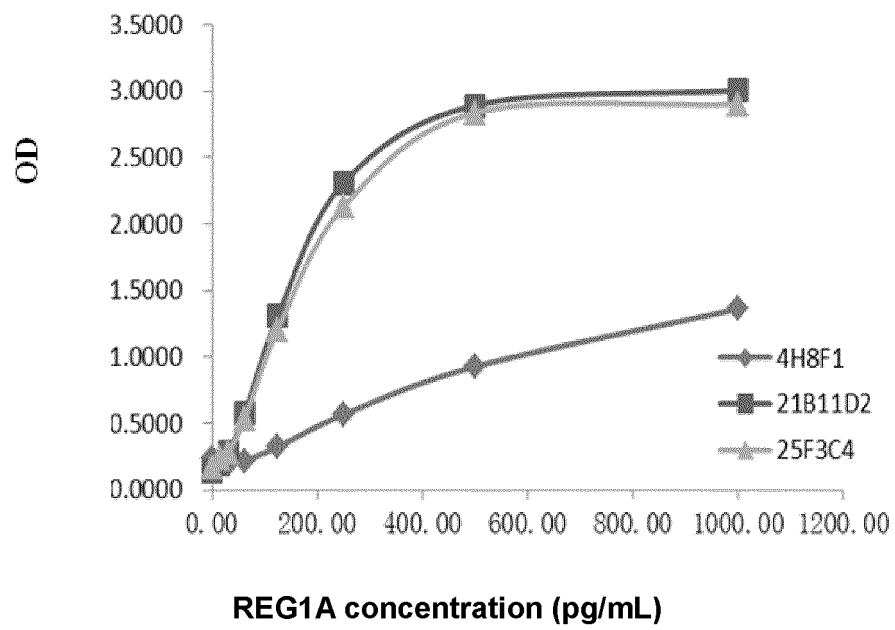
Fig. 1

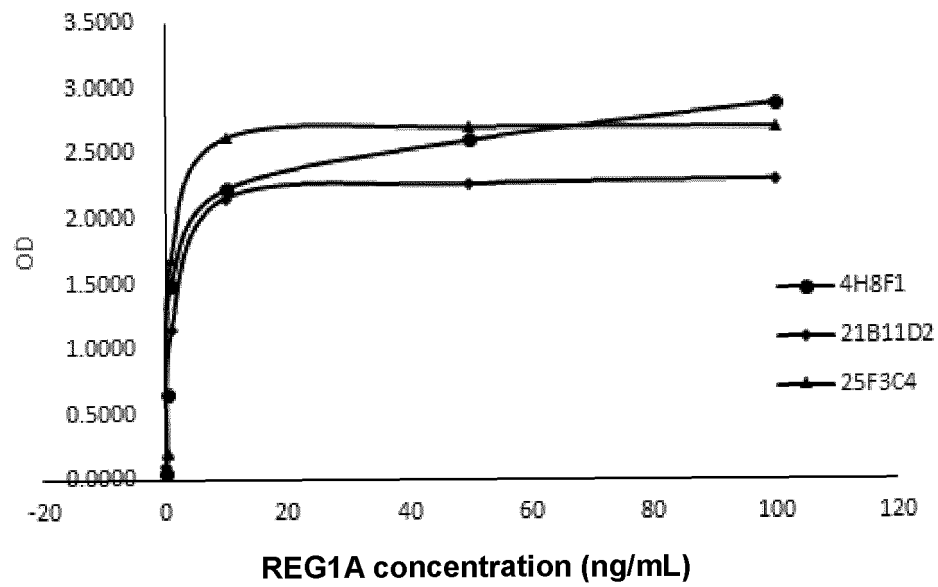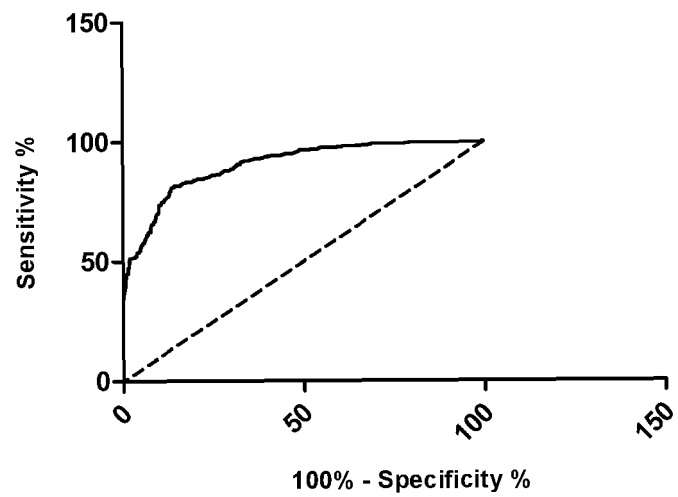REG1A concentration (pg/mL)

Fig. 2

REG1A concentration (ng/mL)

Fig. 3

100% - Specificity %

4-1
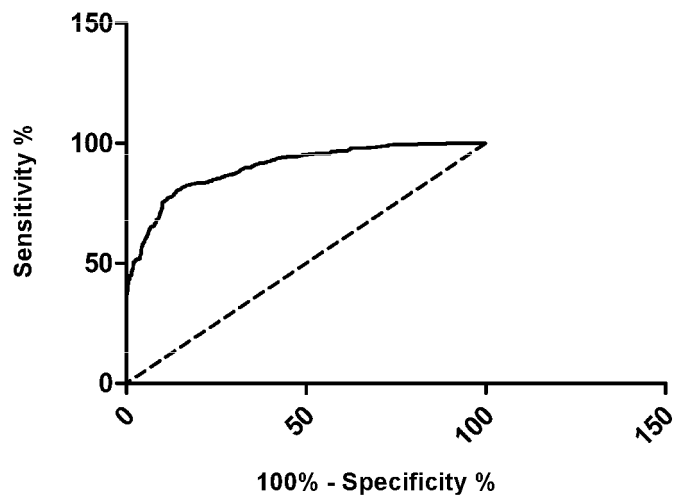
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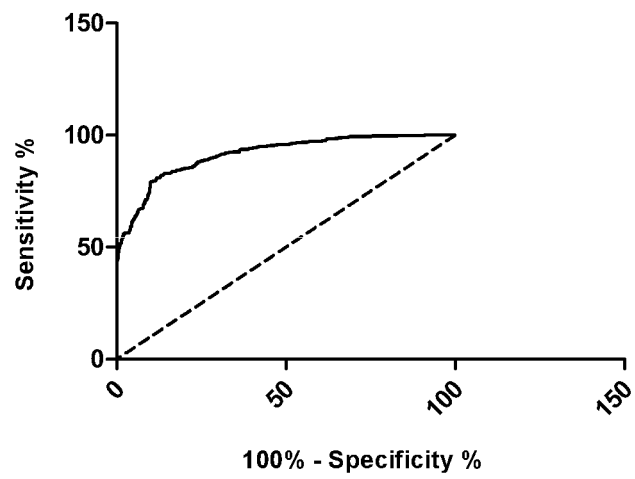
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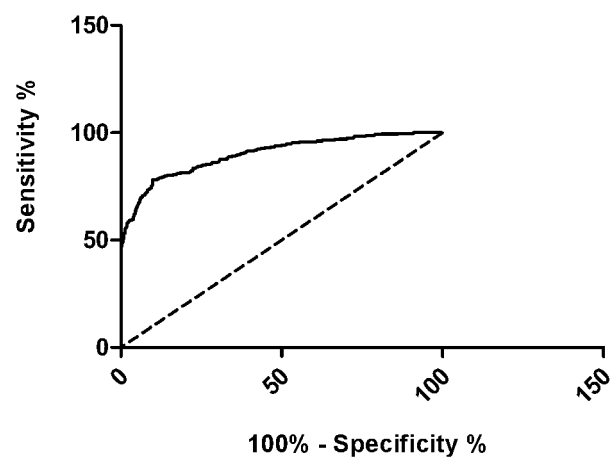
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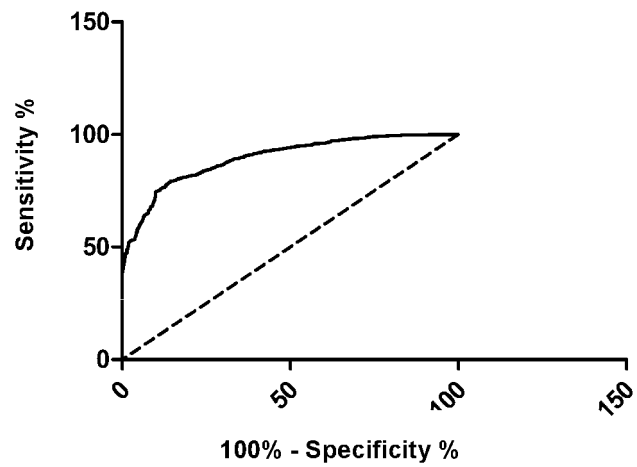
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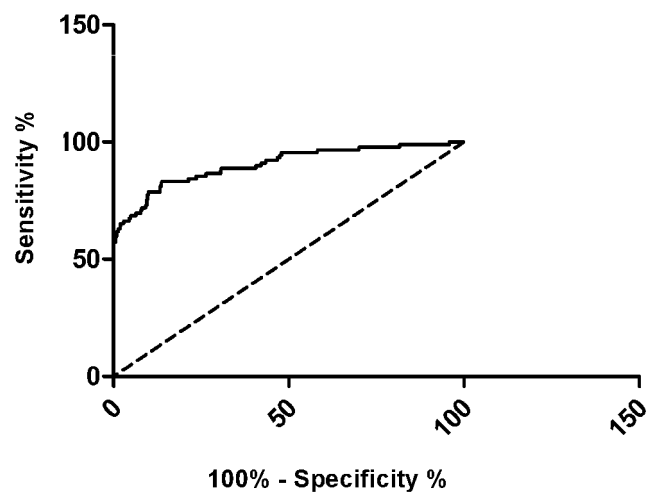
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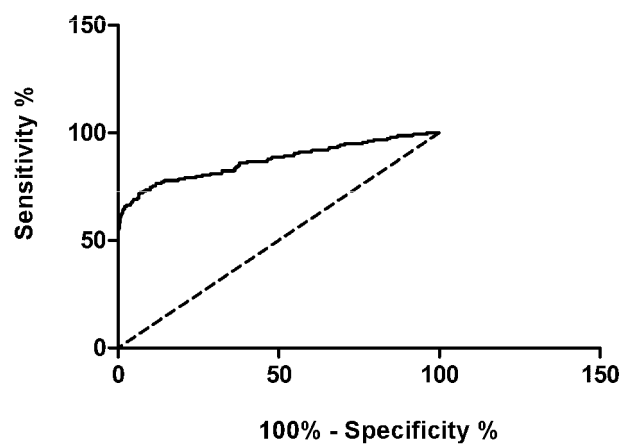
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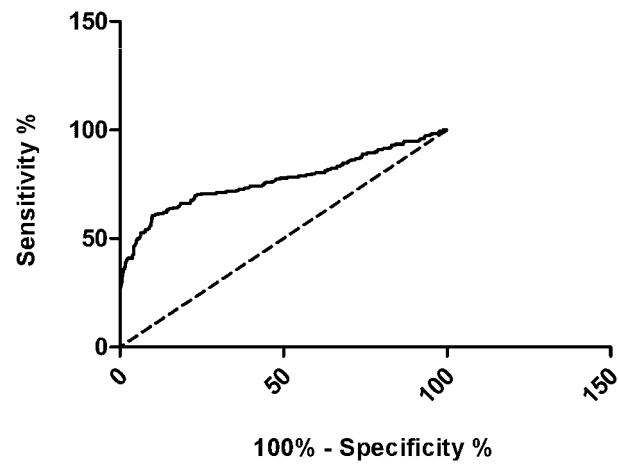
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4-9
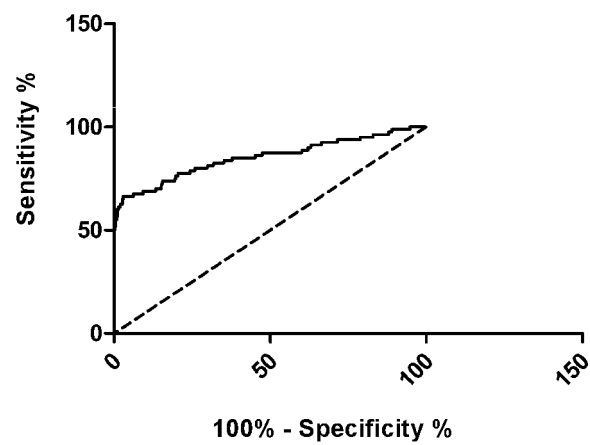
4-10
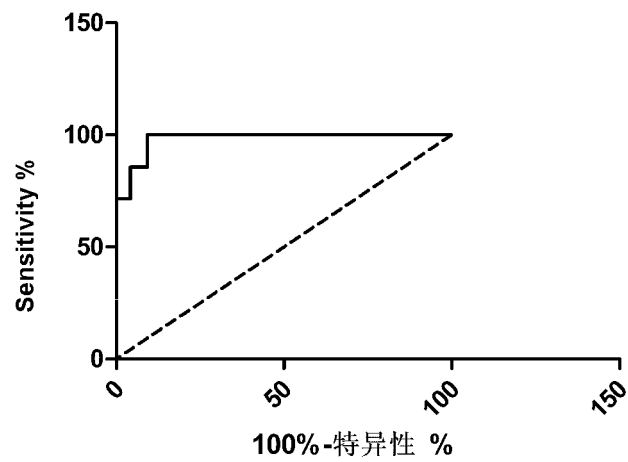
4-11

Fig. 4

Fig. 5

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2022/103719

A.   CLASSIFICATION OF SUBJECT MATTER

C07K 16/18(2006.01)i; G01N 33/68(2006.01)i; G01N 33/577(2006.01)i; G01N 33/574(2006.01)i
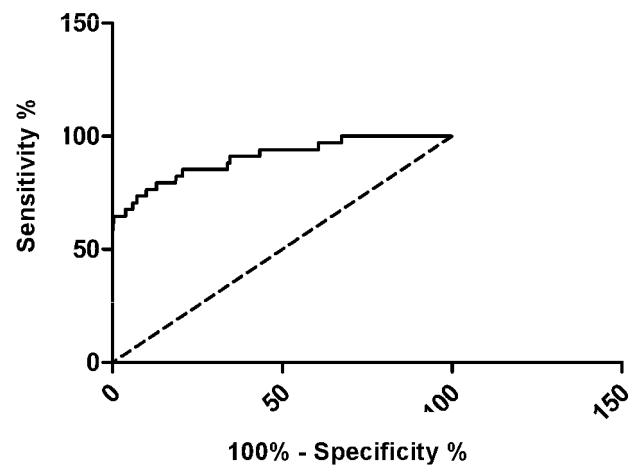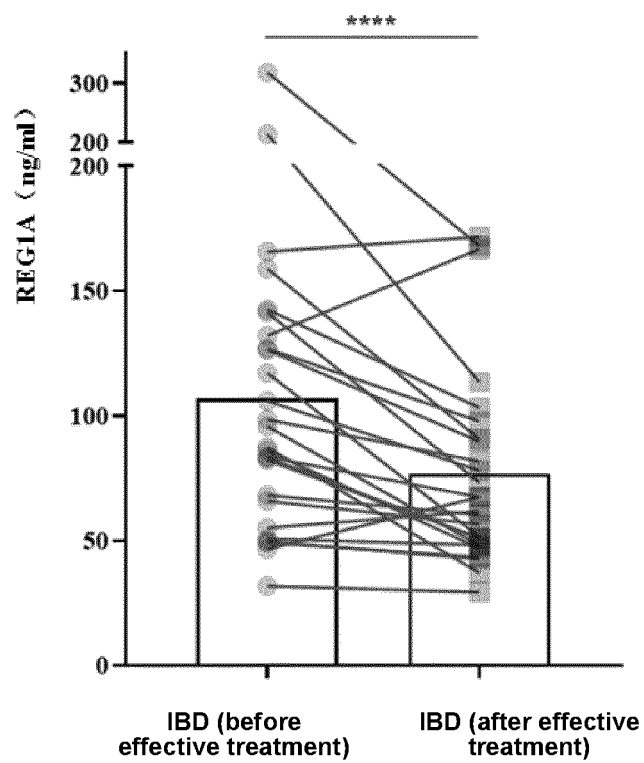
According to International Patent Classification (IPC) or to both national classification and IPC

B.   FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C07K, G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CNABS, DWPI, SIPOABS, CNKI, 万方 WANFANG, ISI Web of Science, BAIDU: 再生胰岛衍生蛋白1α, 胰岛再生原蛋白1α, 单克隆抗体, regenerating family member 1 alpha, REG1A, regenerating gene, REG, monoclonal antibody, 4H8F1, 12F12A10 等; GenBank, EMBL, 中国专利生物序列检索系统, China Patent Biological Sequence Retrieval System, STN, 全文库和检索 的序列: SEQ ID NOs: 1-20, Full-text database and searched sequences: SEQ ID NOs: 1-20.

C.   DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
PX	CN 113248609 A (SHENZHEN SHENGBOER LIFE SCIENCE AND TECHNOLOGY CO., LTD.) 13 August 2021 (2021-08-13) see claims 1-37, and description, paragraphs 6-22	1-35
A	CN 110456072 A (SHENZHEN SHENGBOER LIFE SCIENCE AND TECHNOLOGY CO., LTD.) 15 November 2019 (2019-11-15) see claims 7-10, description, paragraphs 13-41, and embodiments 1-2	1-35
A	CN 107674884 A (SHANGHAI SUCHUANG DIAGNOSIS PRODUCTS CO., LTD.; FUDAN UNIVERSITY) 09 February 2018 (2018-02-09) see claim 6, embodiments 1-5	1-35
A	JP 2010046057 A (AKITA UNIVERSITY) 04 March 2010 (2010-03-04) see claims 1-19	1-35
A	CN 107533062 A (UNIVERSITY LONDON QUEEN MARY) 02 January 2018 (2018-01-02) see claims 1-32	1-35

☑ Further documents are listed in the continuation of Box C.   ☑ See patent family annex.

* Special categories of cited documents: "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier application or patent but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
20 July 2022	**10 October 2022**

Name and mailing address of the ISA/CN	Authorized officer
China National Intellectual Property Administration (ISA/ CN) **No. 6, Xitucheng Road, Jimenqiao, Haidian District, Beijing 100088, China**	
Facsimile No. **(86-10)62019451**	Telephone No.

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2022/103719

C.   DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2011127344 A2 (UNIVERSITY OF VIRGINIA PATENT FOUNDATION et al.) 13 October 2011 (2011-10-13) see claims 1-22, embodiments 1-3	1-35
A	EP 0369797 A2 (SHIONOGI & CO.) 23 May 1990 (1990-05-23) see claims 1-12	1-35

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Box No. I	Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

    a. ☑ forming part of the international application as filed:

        ☑   in the form of an Annex C/ST.25 text file.

        ☐   on paper or in the form of an image file.

    b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

    c. ☐ furnished subsequent to the international filing date for the purposes of international search only:

        ☐   in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

        ☐   on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

    [1]   the sequence table actually submitted is an XML document of an ST.26 standard.

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INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.

PCT/CN2022/103719

Patent document cited in search report			Publication date (day/month/year)	Patent family member(s)			Publication date (day/month/year)
CN	113248609	A	13 August 2021	None			
CN	110456072	A	15 November 2019	None			
CN	107674884	A	09 February 2018	None			
JP	2010046057	A	04 March 2010	None			
CN	107533062	A	02 January 2018	IL	253710	D0	28 September 2017
				BR	112017016808	A2	03 April 2018
				US	2022042998	A1	10 February 2022
				MX	2017009998	A	01 February 2018
				JP	2018504609	A	15 February 2018
				GB	201501930	D0	25 March 2015
				EP	3254111	A1	13 December 2017
				CL	2017001972	A1	23 March 2018
				WO	2016124947	A1	11 August 2016
				SG	11201706223V	A	30 August 2017
				US	2019204323	A1	04 July 2019
				AU	2016214140	A1	17 August 2017
				KR	20170129118	A	24 November 2017
				CA	2974775	A1	11 August 2016
				HK	1247276	A1	21 September 2018
WO	2011127344	A2	13 October 2011	US	2013130965	A1	23 May 2013
EP	0369797	A2	23 May 1990	KR	900007440	A	01 June 1990
				JP	H02265498	A	30 October 1990
				HU	895916	D0	28 February 1990
				CA	2003042	A1	16 May 1990
				NZ	231392	A	25 September 1991
				DK	571189	D0	15 November 1989
				AU	4472689	A	24 May 1990

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REFERENCES CITED IN THE DESCRIPTION

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Patent documents cited in the description

- CN 202110798131 **[0001]**